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Most patients with advanced prostate cancer have bone metastases. These metastases contribute significantly to the morbidity and mortality associated with advanced prostate cancer. Unfortunately, our knowledge of how and why prostate cancer metastasizes to bone is limited. The standard treatment for patients with advanced prostate cancer is androgen deprivation therapy. Treatment with androgen deprivation therapy leads to an increase in bone turnover as indicated by the loss of bone mineral density and the increase in markers of bone turnover in patients on treatment. This increase in bone turnover may result in an increase in bone metastases in patients with advanced prostate cancer. We have developed a mouse model which mimics the clinical scenario where men treated with androgen deprivation therapy develop bone metastases. Furthermore, we have used this model to test the effectiveness of zoledronic acid, a potent inhibitor of bone resorption, as a preventative treatment for prostate cancer bone metastases. Our data indicates that prevention of bone resorption by agents such as zoledronic acid beginning at onset of androgen deprivation therapy may result in significantly fewer bone metastases in patients with advanced prostate cancer.

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INTRODUCTION

Most patients with advanced prostate cancer have bone metastases. These metastases contribute significantly to the morbidity and mortality associated with advanced prostate cancer. Unfortunately, our knowledge of how and why prostate cancer metastasizes to bone is limited. The standard treatment for patients with advanced prostate cancer is androgen deprivation therapy. Treatment with androgen deprivation therapy leads to an increase in bone turnover as indicated by the loss of bone mineral density and the increase in markers of bone turnover in patients on treatment. This increase in bone turnover may result in an increase in bone metastases in patients with advanced prostate cancer. We have developed a mouse model which mimics the clinical scenario where men treated with androgen deprivation therapy develop bone metastases. Furthermore, we have used this model to test the effectiveness of zoledronic acid, a potent inhibitor of bone resorption, as a preventative treatment for prostate cancer bone metastases. Our data indicates that prevention of bone resorption by agents such as zoledronic acid beginning at onset of androgen deprivation therapy may result in significantly fewer bone metastases in patients with advanced prostate cancer.

BODY

Task 1 of the approved statement of work was to test the effects of androgen ablation by orchiectomy on the development and progression of human prostate tumor cells in the bones of male nude mice following intracardiac inoculation.

Athymic male nude mice were orchiectomized and four weeks later we performed bone histomorphometry to assess the effects of hypogonadism on the bones of mice without tumor. Hypogonadal animals had significantly less trabecular bone compared to intact animals. This was associated with a significant increase in osteoclast numbers. Thus, androgen deprivation via orchiectomy resulted in bone loss and increased osteoclastic bone resorption in this mouse model.

Using this model of hypogonadism, four prostate cancer cell lines, DU-145, LN.CaP, LN.CaP-LN3, PC-3 and one bladder cancer cell line, TSU-PR1 have been tested for the ability to form bone metastases following intra-cardiac inoculation. Results with each cell line are outlined below. In addition, multiple time points for orchiectomy and tumor cell inoculation have been tested. The best results with respect to an effect on bone and on bone metastases were seen when mice underwent orchiectomy or sham surgery at day 0 and tumor cell inoculation four weeks later. This scenario was used for all of the studies described below. This mimics the clinical situation in which tumor cells seed to bone in a patient who has been hypogonadal for a longer period and who has high bone turnover induced by this condition.

DU-145. Mice were inoculated with DU-145 prostate cancer cells four weeks after orchiectomy or sham surgery. We then performed radiography, measurements of bone mineral density and took serum samples at regular intervals. Hypogonadal animals had significantly lower bone mineral density than intact animals. Although there was some initial evidence of osteolytic activity in the long bones of these mice, they died from brain tumors before they developed bone metastases. It should be noted that DU-145 cells were originally isolated from a brain metastases in a prostate cancer patient.

LN.CaP. Mice were inoculated with LN.CaP cells, a prostate cancer cell line originally isolated from a lymph node metastases, 4 weeks after orchiectomy or sham surgery. Mice were followed by radiography, measurements of bone mineral density. Serum samples were taken at regular intervals. Hypogonadal animals had significantly lower bone mineral density however, no evidence of bone metastases was seen by radiography after one year.

LN.CaP-LN3. LN.CaP-LN3 cells are a derivative of the parental LN.CaP cells that have been reported to be more metastatic than the parental cells. These cells were also inoculated into the left cardiac ventricle four weeks after orchiectomy or sham surgery. Significantly lower bone mineral density was also observed in hypogonadal mice compared to sham treated mice inoculated with LN.CaP-LN3 cells. These cells do not make bone metastases in this model.

PC-3. As with the other cell line, mice were inoculated with PC-3 prostate cancer cells four weeks after orchiectomy or sham surgery. PC-3 cells, originally derived from a prostate cancer metastasis to bone, reliably formed osteolytic lesions in this model (Padalecki et al., ASBMR 2001). Hypogonadism resulted in a loss of bone mineral density compared to sham treated mice (Appendix 1, Figure 1) . In contrast to results with the other prostate cancer cell lines tested, hypogonadal mice bearing PC-3 tumor cells had more bone metastases and these occurred sooner than in intact mice bearing PC-3 cells. While these lesions were predominantly osteolytic in nature, we have observed occasional osteoblastic metastases.

TSU-Pr1. TSU-PR1, originally thought to be a prostate cancer cell line derived from a lymph node metastasis, has recently been shown to be bladder cancer in origin (van Bokhoven et al., 2001). Despite this, we have continued to work with TSU-PR1 cells because bladder cancer is a malignancy of urologic origin and may have some similarities with prostate cancer and because this has proven to be a useful model for bone metastases in our laboratory. TSU-PR1 cells were inoculated via the left cardiac ventricle, four weeks after orchiectomy or sham surgery. Again, hypogonadism resulted in significantly less bone mineral density than in sham treated mice (Appendix 2, Figure 2). Both hypogonadal and intact mice bearing TSU-PR1 cells developed bone metastases. However, these occurred sooner and more frequently in hypogonadal animals (Appendix 3, Figure 3). These bone metastases are interesting for the purposes of this study since they are of a mixed osteolytic-osteoblastic nature. Initially, lesions appear to be bone destructive or osteolytic but as they progressed we observed new bone formation. This would suggest that osteoclastic bone resorption and new bone formation are coupled in this model. These results may help explain why markers of bone resorption are increased in patients with prostate cancer while prostate cancer bone metastases are primarily osteoblastic (or mixed) in nature. Since this model may provide insight into the mechanisms of mixed bone metastases, we continue to utilize it in our work.

Task 2. To determine if inhibition of the increased bone resorption induced by androgen deprivation will reduce the development and progression of prostate cancer metastases to bone.

Our initial work to determine the effects of increased bone resorption induced by androgen deprivation has been performed using the PC-3 prostate cancer bone metastasis model described above. Mice underwent orchiectomy or sham surgery at day 0. Four weeks later, when we know bone turnover would be increased, we inoculated the mice with PC-3 cells via the left cardiac ventricle. Mice were treated with the bisphosphonate, zoledronic acid, a potent inhibitor of osteoclastic bone resorption, in a preventative manner, beginning at the time of surgery.

Zoledronic acid significantly increased the bone mineral density at the tibia and femur regardless of surgical treatment (Appendix 4, Figure 4). In addition, hypogonadal animals treated with vehicle had significantly lower bone mineral density at these sites when compared to intact animals treated with vehicle (figure 4). Zoledronic acid also resulted in fewer bone metastases in hypogonadal animals compared to hypogonadal

animals treated with vehicle (Appendix 5, Figure 5). Histomorphometric analysis indicated that hypogonadal mice treated with vehicle had increased tumor burden in bone compared with intact mice treated with vehicle. Treatment with zoledronic acid significantly reduced the tumor burden in bone in hypogonadal mice (Appendix 6, Figure 6 and Appendix 7, Figure 7). In addition to an increase in tumor burden, hypogonadism also resulted in an increase in osteoclast number at the tumor bone interface compared to intact mice treated with vehicle. This was significantly reduced by treatment with zoledronic acid (Appendix 8, Figure 8). In addition, zoledronic acid treatment significantly improved the survival of hypogonadal mice bearing PC-3 tumor cells (Appendix 9 Figure 9). However, no difference in the number of extraskeletal metastases was observed in mice regardless of gonadal status or drug treatment.

Experiments are currently underway to determine the effects on bone metastases of treating hypogonadal or intact mice with testosterone beginning at the time of surgery.

We are also currently performing experiments with the TSU-PR1 model to determine the effects of using zoledronic acid to inhibit osteoclastic bone resorption in a model of mixed osteolytic/osteoblastic bone metastases.

KEY RESEARCH ACCOMPLISHMENTS:

- Hypogonadism results in a loss of bone mineral density at the tibia and femur in athymic male nude mice.
- Hypogonadism results in a significant decrease in trabecular bone area and an increase in osteoclastic bone resorption in male nude mice.
- Hypogonadism results in an increase in bone metastases by PC-3 prostate cancer cells in male nude mice.
- Hypogonadism accelerates the appearance of bone metastases by TSU-Pr1 bladder cancer cells in male nude mice.
- Bisphosphonate inhibition of osteoclastic bone resorption results in an increase in bone mineral density regardless of gonadal status in male nude mice.
- Zoledronic acid treatment significantly reduces the number of skeletal metastases by PC-3 cells in this model.
- Zoledronic acid treatment increases the survival of hypogonadal mice bearing PC-3 tumor cells in this model.

REPORTABLE OUTCOMES:

Manuscript in progress for submission to Nature Medicine

Abstracts related to this work:

Padalecki, S.S., Carreon, M., Grubbs, B., Cui, Y. and T.A. Guise (2001) Bisphosphonates prevent bone loss and the development of prostate cancer bone metastases associated with androgen deprivation. American Association for Cancer Research New Discoveries in Prostate Cancer Diagnosis and Treatment Special Conference. (Poster Presentation)

Padalecki, S.S., Carreon, M., Grubbs, B., Cui, Y. and T.A. Guise (2002) Androgen deprivation enhances bone loss and prostate cancer metastases to bone: prevention by zoledronic acid. Third North American Symposium on Skeletal Complications of Malignancy. (Oral Presentation)

Padalecki, S.S., Carreon, M., Grubbs, B., Cui, Y. and T.A. Guise (2002) Androgen deprivation enhances bone loss and prostate cancer metastases to bone: prevention by zoledronic acid. San Antonio Cancer Institute's 12th Annual Symposium on Cancer Research in San Antonio.

Padalecki, S.S., Carreon, M., Grubbs, B., Cui, Y. and T.A. Guise. (2002) Androgen deprivation causes bone loss and increased prostate cancer metastases to bone: prevention by zoledronic acid. American Society for Bone and Mineral Research Annual Meeting.

Other funding applied for based on work supported by this award:

2001 CaPCURE Foundation Young Investigator Award

CONCLUSIONS:

Thus far, this work has confirmed that hypogonadism results in osteoclastic bone resorption and bone loss in athymic male nude mice as it does in men. Furthermore, the work with the PC-3 cell line indicates that androgen deprivation, the standard treatment for patients with advanced prostate cancer increases bone resorption and bone metastases. In addition, using TSU-PR1 bladder cancer cells in this model resulted in an increase in bone metastases as well. Furthermore, inhibition of osteoclastic bone resorption led to a decrease in bone metastases by PC-3 cells in this model.

What does this mean for patients with prostate cancer? It seems likely that the inhibition of osteoclastic bone resorption using bisphosphonates such as zoledronic acid at the onset of androgen deprivation therapy may reduce the osteoporosis associated with hypogonadism and decrease the incidence of prostate cancer metastases to bone.

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Padalecki, S.S.,. Johnson-Pais, T.L., Weldon, K.S., Reveles, X., Buller, C.L., Grubbs, B., Cui, Y., Dallas, M., Leach, R.J. and T.A. Guise (2001) Chromosome 18 suppresses prostate cancer metastases. J Bone Min Res 16: SU087.

Van Bokhoven A, Varella-Garcia M, Korch C, Miller GJ. TSU-Pr1 and JCA-1 cells are derivative of T24 bladder carcinoma cells and are not of prostatic origin. Cancer Res 2001; 61: 6340-6344.

APPENDICES

Appendix 1. Figure 1

Appendix 2. Figure 2

Appendix 3. Figure 3

Appendix 4. Figure 4

Appendix 5. Figure 5

Appendix 6. Figure 6

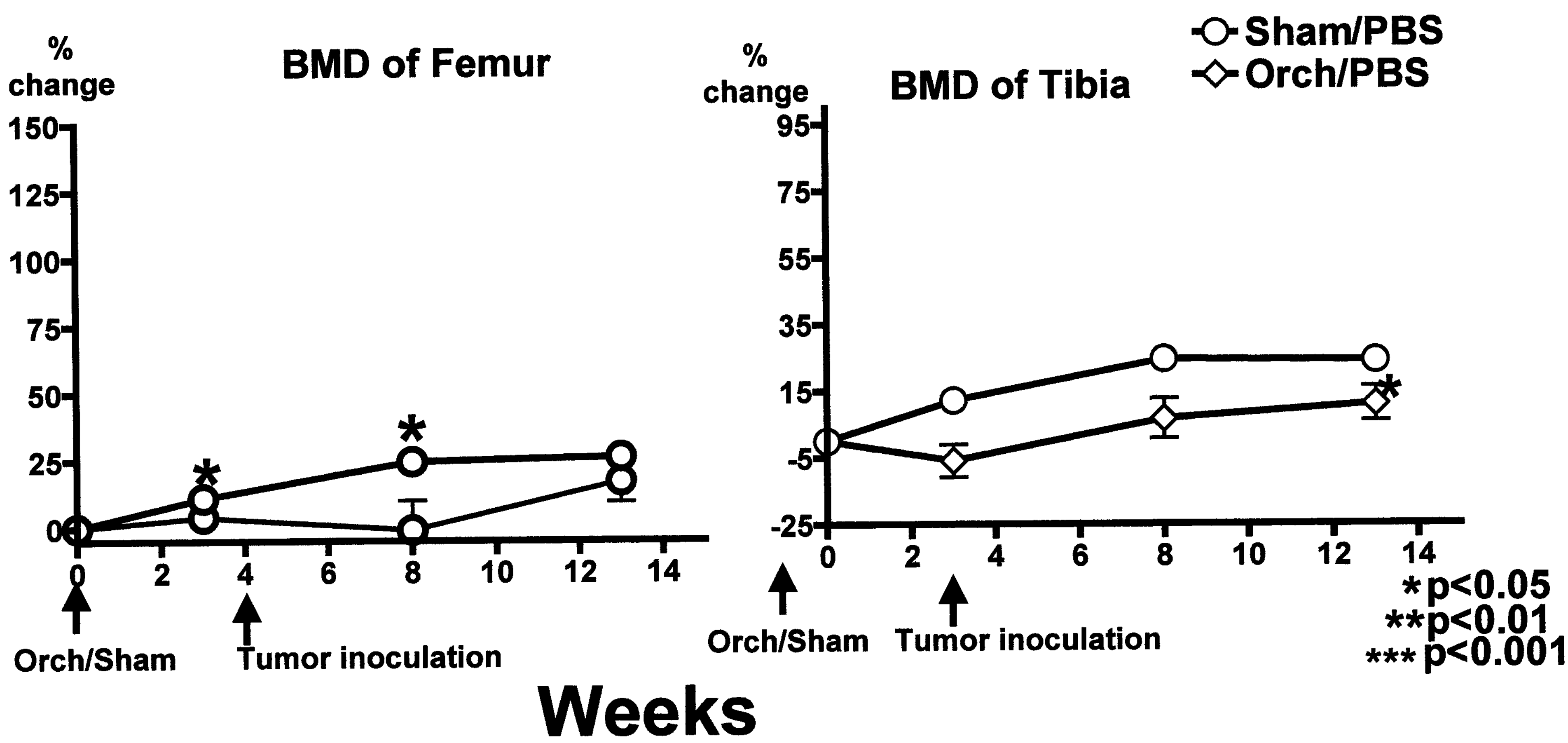
Appendix 7. Figure 7

Appendix 8. Figure 8

Appendix 9. Figure 9

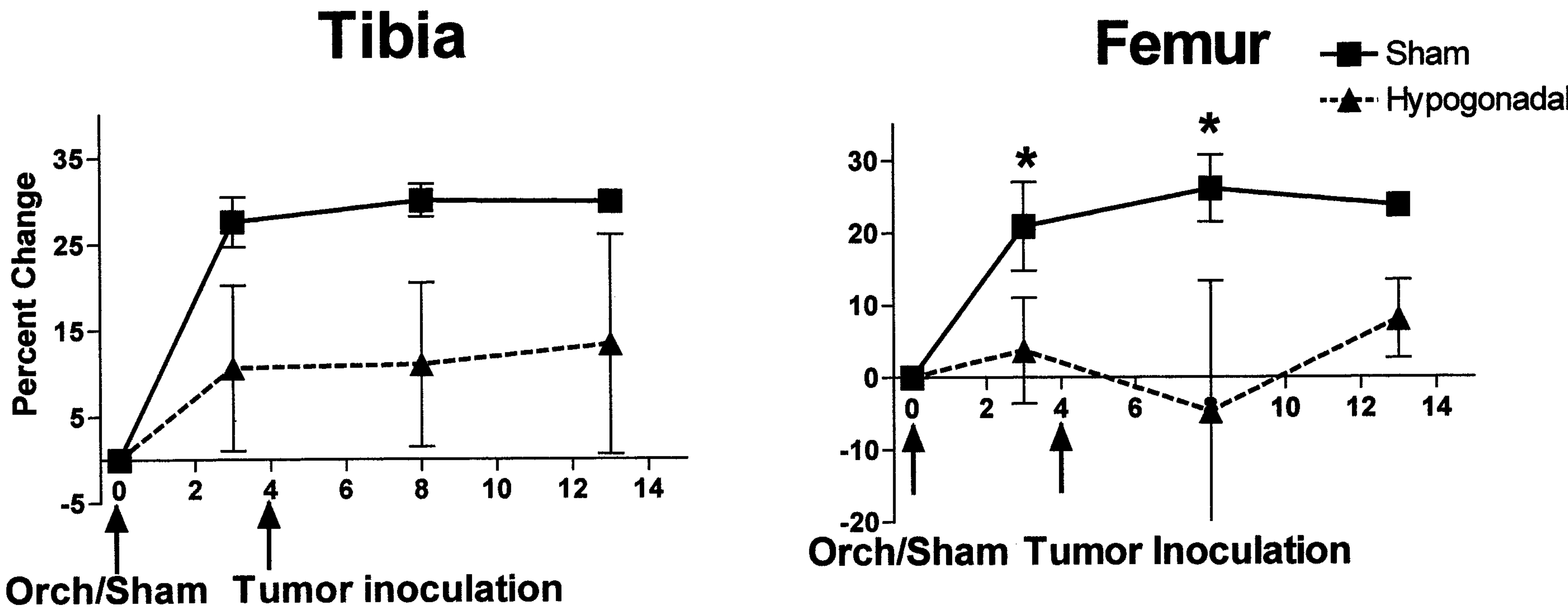
Appendix 1

Figure 1: Hypogonadism in mice inoculated with PC-3 cells resulted in a loss of bone mineral density compared to sham treated mice inoculated with PC-3 cells.



Appendix 2

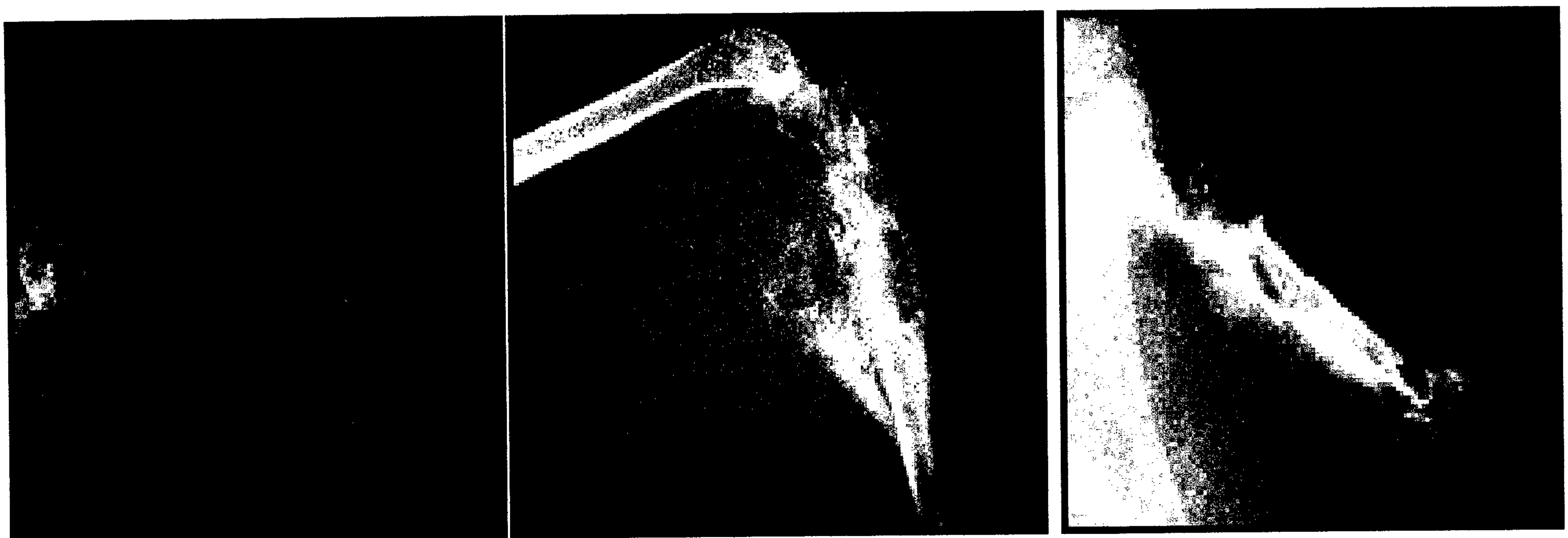
Figure 2: Hypogonadism in mice inoculated with TSU-PR1 cells resulted in a loss of bone mineral density in the femur compared to sham treated mice inoculated with TSU-PR1 cells



* $p<0.05$

Appendix 3

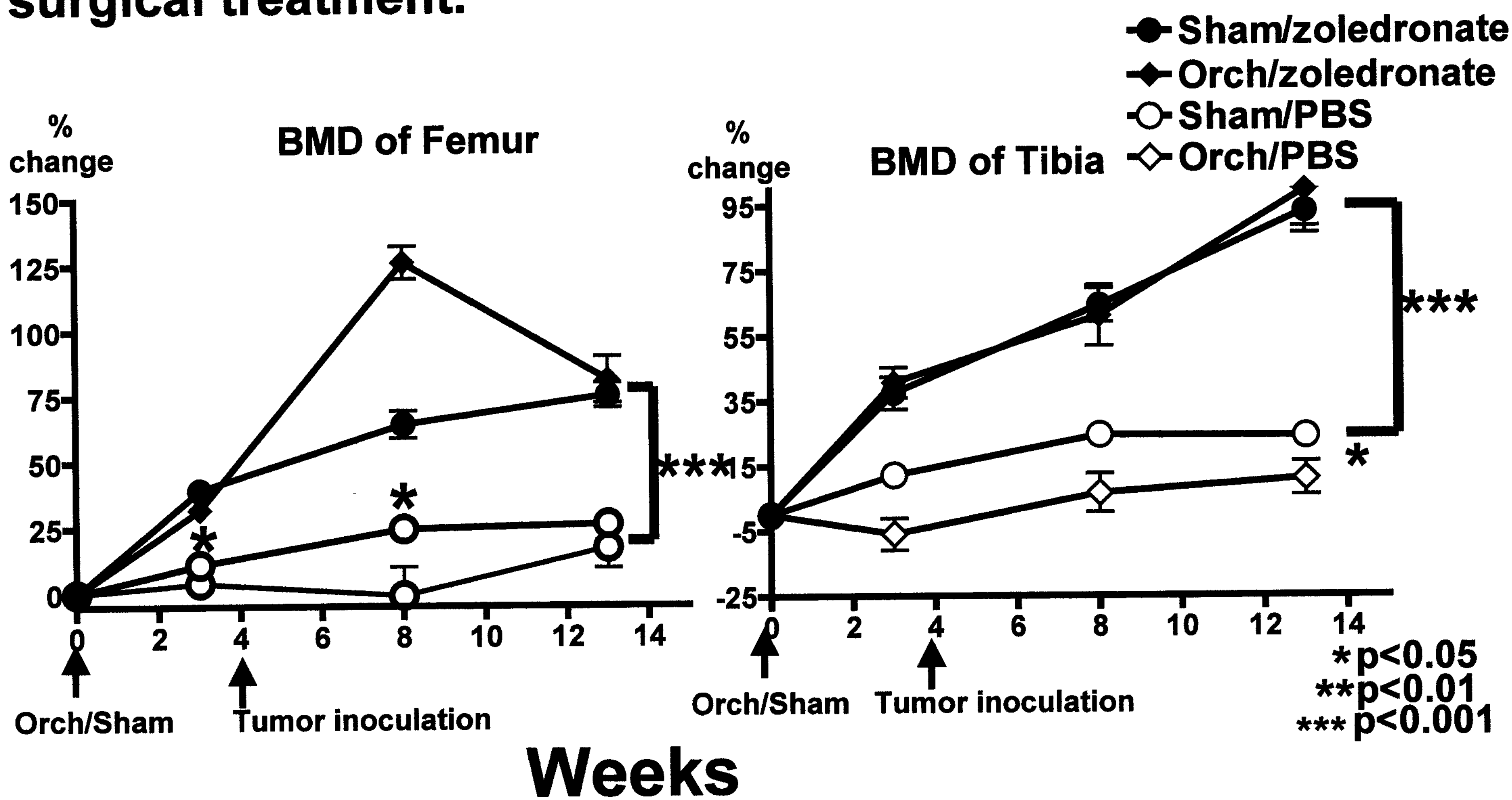
Figure 3: Representative X-rays from intact and hypogonadal mice inoculated with TSU-PR1 cells. Note that both groups develop bone metastases that are mixed in nature. However, intact mice develop them later (18 weeks post tumor Inoculation as indicated by the time point below the Xrays) than hypogonadal mice (9 weeks post tumor inoculation).



Intact (18wks) **Hypogonadal (9 wks)**

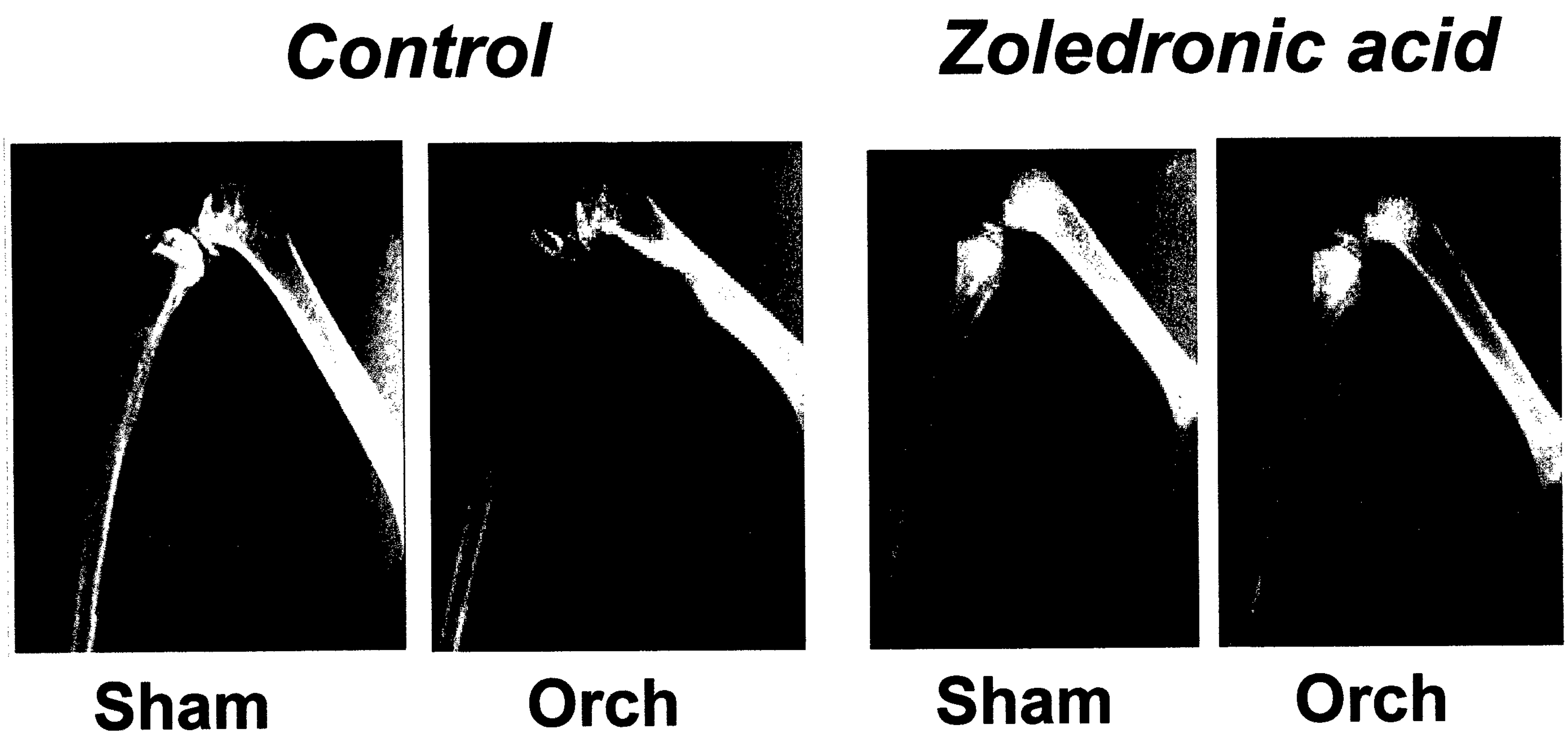
Appendix 4

Figure 4: Hypogonadism in mice inoculated with PC-3 cells resulted in a loss of bone mineral density compared to sham treated mice inoculated with PC-3 cells. Zoledronic acid significantly increased the bone mineral density regardless of surgical treatment.



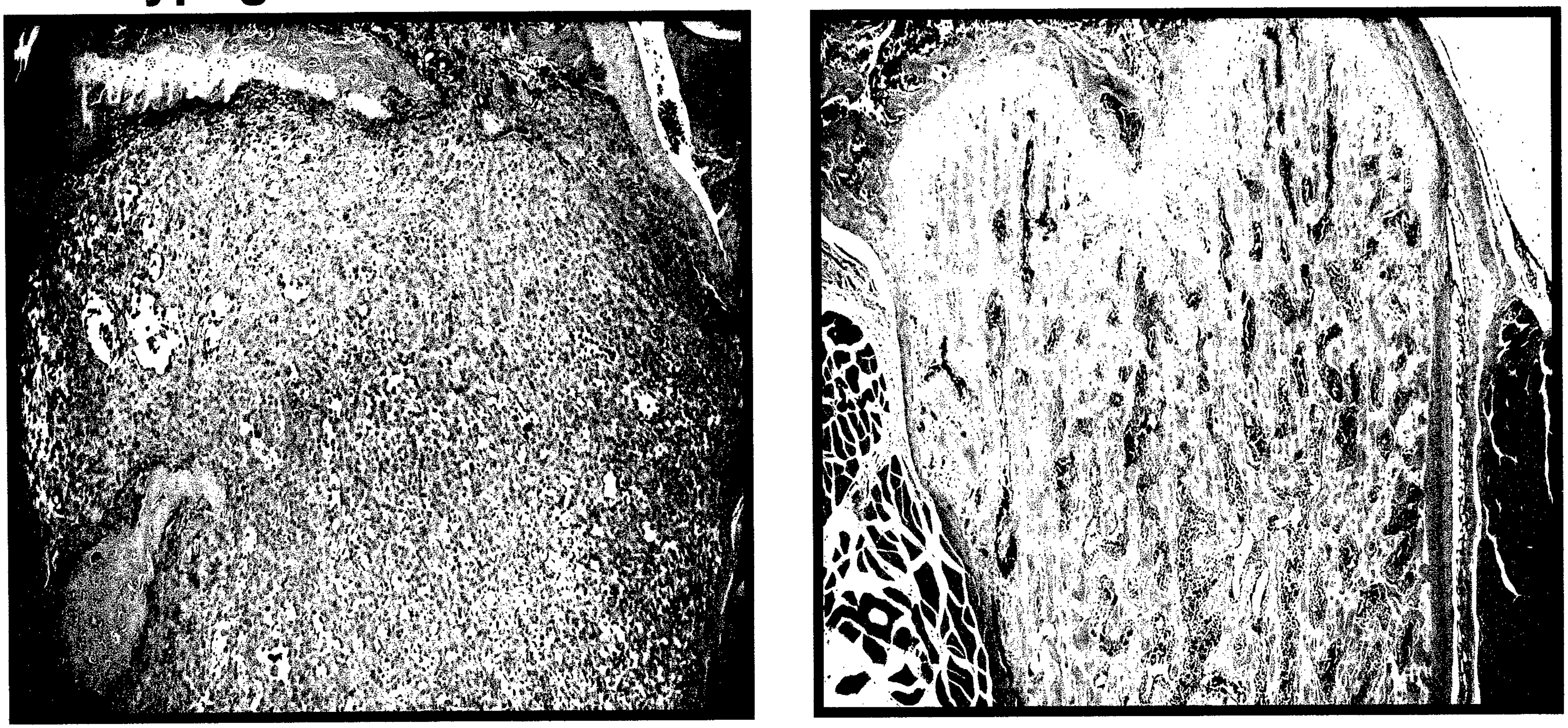
Appendix 5

Figure 5: Hypogonadism in mice inoculated with PC-3 cells and treated with vehicle resulted increased bone metastases compared to sham treated mice receiving the same treatment. Zoledronic acid resulted in fewer bone metastases evident by Xray.



Appendix 6

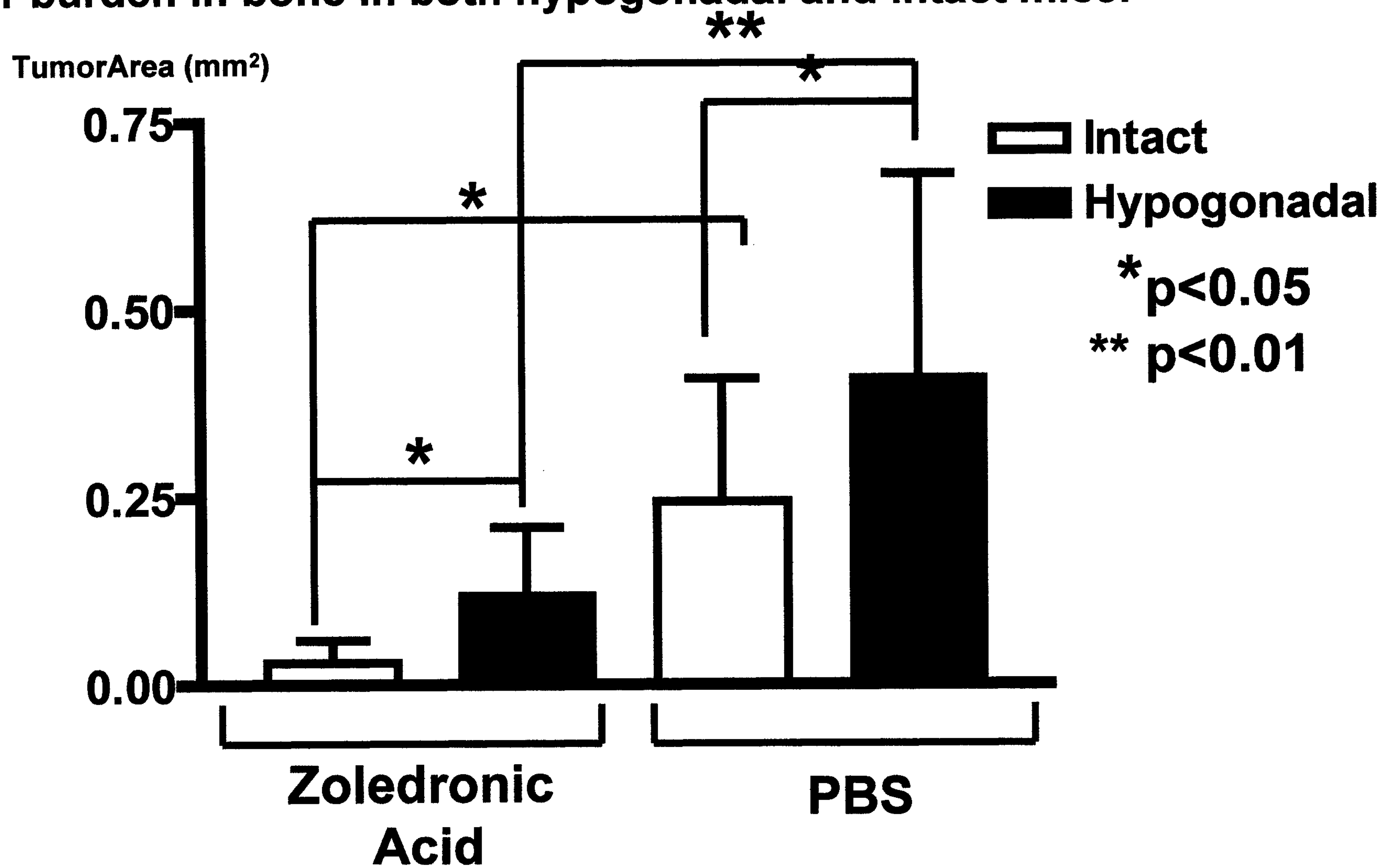
Figure 6: Representative histological sections from the hindlimbs of hypogonadal mice inoculated with PC-3 cells and treated with either vehicle (left) or zoledronic acid (right). Note the extensive bone destruction caused by tumor cells in the hypogonadal mouse treated with vehicle.



Vehicle **Zoledronic Acid**

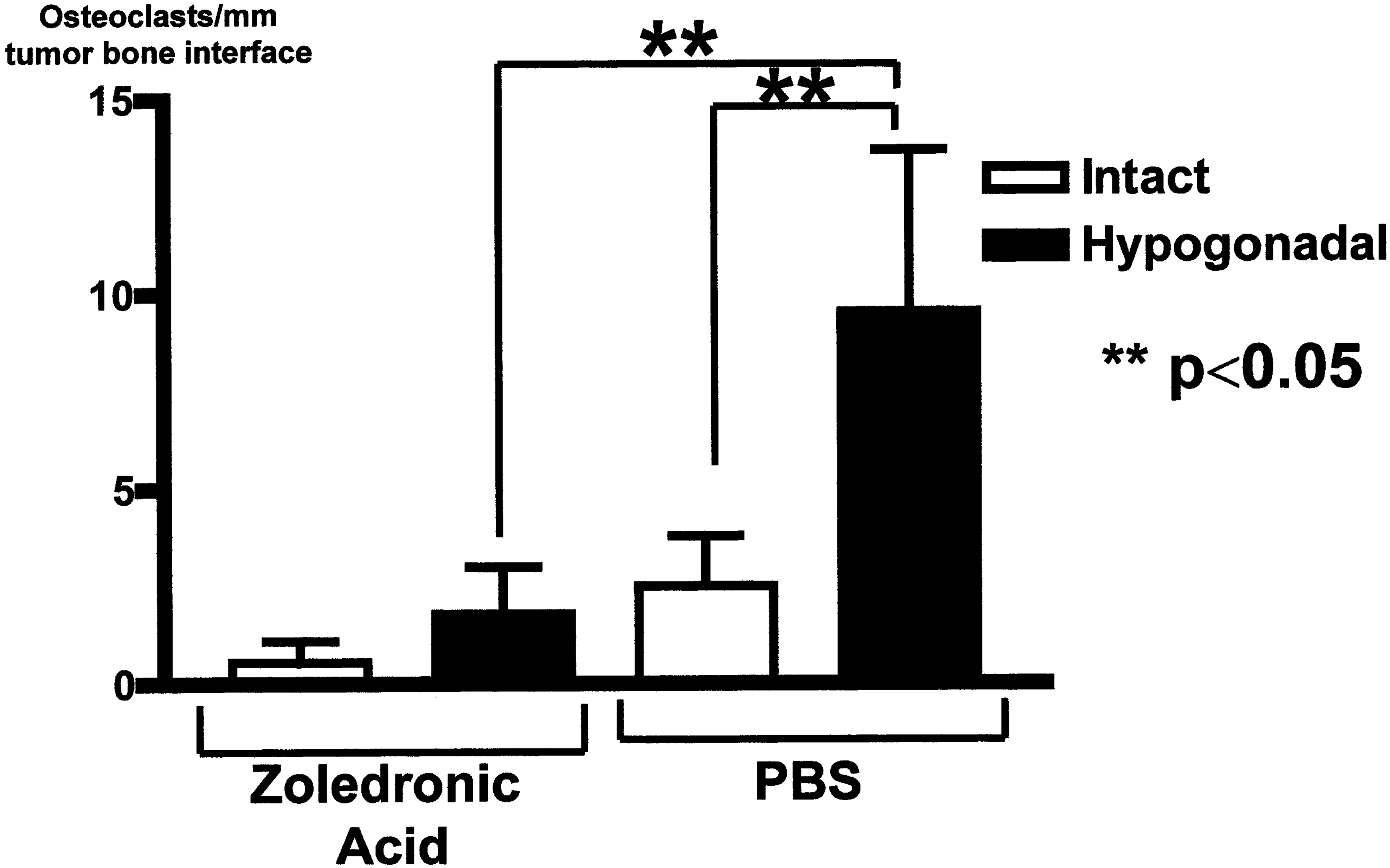
Appendix 7

Figure 7: Histomorphometric analysis of tumor burden in bone. Hypogonadal mice inoculated with PC-3 cells and treated with vehicle (right) have significantly more tumor in bone than intact animals receiving the same treatment. Treatment with zoledronic acid significantly reduced tumor burden in bone in both hypogonadal and intact mice.



Appendix 8

Figure 8: Histomorphometric analysis of osteoclast number at the tumor bone interface. Hypogonadal mice inoculated with PC-3 cells (receiving PBS) have a significant increase in the number of osteoclasts at the tumor-bone interface than intact animals receiving the same treatment. Treatment with zoledronic acid significantly reduced the number of osteoclasts at the tumor-bone interface.



Appendix 9

Figure 9: Zoledronic acid treatment significantly improved the survival of hypogonadal mice inoculated with PC-3 cells compared to all other groups.